

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/62, A61K 39/29, C07K 14/18	A2	(11) International Publication Number: WO 97/01640 (43) International Publication Date: 16 January 1997 (16.01.97)
<p>(21) International Application Number: PCT/EP96/02764</p> <p>(22) International Filing Date: 20 June 1996 (20.06.96)</p> <p>(30) Priority Data: 9513261.9 29 June 1995 (29.06.95) GB</p> <p>(71) Applicant (for all designated States except US): SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): CABEZON SILVA, Teresa [CL/BE]; SmithKline Beecham Biological S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). MOMIN, Patricia, Marie [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). GARÇON, Nathalie, Marie-Joséphé, Claude [FR/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE).</p> <p>(74) Agent: WEST, Vivien; SmithKline Beecham, Corporate Intellectual Property, SB House, Great West Road, Brentford, Middlesex TW8 9BD (GB).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p>
<p>(54) Title: VACCINES AGAINST HEPATITIS C</p> <p>(57) Abstract</p> <p>A vaccine composition comprises QS21,3 De-O-acylated monophosphoryl lipid A (3D-MPL), an oil in water emulsion, wherein the oil in water emulsion has the following composition: a metabolisable oil, such as squalene, alpha tocopherol and tween 80, and at least one immunogen selected from the group consisting of (a) a hepatitis C virus core protein or an immunogenic derivative thereof, and (b) a hepatitis C virus envelope protein or an immunogenic derivative thereof.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

VACCINES AGAINST HEPATITIS C

The present invention relates to novel vaccine formulations, to methods of their production and to their use in medicine.

5 3 De-O-acylated monophosphoryl lipid A is known from GB2 220 211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in International Patent Application No. 92/116556.

10 QS21 is a Hplc purified non toxic fraction of a saponin from the bark of the South American tree Quillaja Saponaria Molina and its method of its production is disclosed (as QA21) in US patent No. 5,057,540.

Oil in water emulsions per se are known in the art, and have been suggested to be useful as adjuvant compositions (EPO 399843).

15 Hepatitis C virus is described in EP-A-0 318 216. A particular antigenic protein of hepatitis C virus has been designated the core protein and is described by, for example, Delisse et al., J. Hepatology, 1991;13 (Suppl. 4): S20-S23 (for genotype 1b). Particular envelope proteins of hepatitis C virus have been designated E1 and E2 and are described by, for example, Grakoui et al., 1993, J. Virology 67, 1385-1395; 20 Spacte et al., 1992, Virology 188, 819-830; Matsumia et al., J. Virology 66, 1425-1431, and Kohara et al., 1992, J. Gen. Virol. 73, 2313-2318. A majority of the HCV genotypes identified to date are described by Okamoto Hiroaki and Mishiro Shunji, Intervirology, 1994, 37: 68 et seq.

 The present invention provides a vaccine composition comprising QS21, 3 25 De-O-acylated monophosphoryl lipid A (3D-MPL), an oil in water emulsion, wherein the oil in water emulsion has the following composition: a metabolisable oil, such as squalene, alpha tocopherol and tween 80, and at least one immunogen selected from the group consisting of (a) a hepatitis C virus core protein or an immunogenic derivative thereof, and (b) a hepatitis C virus envelope protein or an immunogenic 30 derivative thereof.

 The term "immunogenic derivative" encompasses any molecule such as a truncated or other derivative of the protein which retains the ability to induce an immune response to the protein following internal administration to a human. Such other derivatives can be prepared by the addition, deletion, 35 substitution, or rearrangement of amino acids or by chemical modifications thereof.

 Immunogenic fragments of the protein, which may be useful in the preparation of subunit vaccines, may be prepared by expression of the

appropriate gene fragments or by peptide synthesis, for example using the Merrifield synthesis (The Peptides, Vol 2., Academic Press, NY, page 3).

The immunogenic derivative of the invention can be a hybrid, that is, a fusion polypeptide containing additional sequences which can carry one or more epitopes for other immunogens. Alternatively, the immunogenic derivative of the invention can be fused to a carrier polypeptide or to another carrier which has immunostimulating properties, as in the case of an adjuvant, or which otherwise enhances the immune response to the protein or derivative thereof, or which is useful in expressing, purifying or formulating the protein or derivative thereof.

The invention also extends to the HCV protein or immunogenic derivative thereof when chemically conjugated to a macromolecule using a conventional linking agent such as glutaraldehyde (Geerlings et al, (1988) J, Immunol. Methods, 106, 239-244).

Proteins and their immunogenic derivatives suitable for use in the present invention can be prepared by expressing DNA encoding said protein or derivative thereof in a recombinant host cell and recovering the product, and thereafter, optionally, preparing a derivative thereof.

A DNA molecule comprising such coding sequence can be synthesized by standard DNA synthesis techniques, such as by enzymatic ligation as described by D.M. Roberts *et al* in Biochemistry 1985, 24, 5090-5098, by chemical synthesis, by *in vitro* enzymatic polymerization, or by a combination of these techniques.

Enzymatic polymerisation of DNA may be carried out *in vitro* using a DNA polymerase such as DNA polymerase I (Klenow fragment) in an appropriate buffer containing the nucleoside triphosphates dATP, dCTP, dGTP and dTTP as required at a temperature of 10⁰-37⁰C, generally in a volume of 50ml or less. Enzymatic ligation of DNA fragments may be carried out using a DNA ligase such as T4 DNA ligase in an appropriate buffer, such as 0.05M Tris (pH 7.4), 0.01M MgCl₂, 0.01M dithiothreitol, 1mM spermidine, 1mM ATP and 0.1mg/ml bovine serum albumin, at a temperature of 4⁰C to ambient, generally in a volume of 50ml or less. The chemical synthesis of the DNA polymer or fragments may be carried out by conventional phosphotriester, phosphite or phosphoramidite chemistry, using solid phase techniques such as those described in 'Chemical and Enzymatic Synthesis of Gene Fragments - A Laboratory Manual' (ed. H.G. Gassen and

A. Lang), Verlag Chemie, Weinheim (1982), or in other scientific publications, for example M.J. Gait, H.W.D. Matthes, M. Singh, B.S. Sproat, and R.C. Titmas, *Nucleic Acids Research*, 1982, 10, 6243; B.S. Sproat and W. Bannwarth, *Tetrahedron Letters*, 1983, 24, 5771; M.D. Matteucci and M.H. Caruthers, *Tetrahedron Letters*, 1980, 21, 719; M.D. Matteucci and M.H. Caruthers, *Journal of the American Chemical Society*, 1981, 103, 3185; S.P. Adams et al., *Journal of the American Chemical Society*, 1983, 105, 661; N.D. Sinha, J. Biernat, J. McMannus, and H. Koester, *Nucleic Acids Research*, 1984, 12, 4539; and H.W.D. Matthes et al., *EMBO Journal*, 1984, 3, 801.

DNA polymers which encode mutants may be prepared by site-directed mutagenesis by conventional methods such as those described by G. Winter *et al* in *Nature* 1982, 299, 756-758 or by Zoller and Smith 1982; *Nucl. Acids Res.*, 10, 6487-6500, or deletion mutagenesis such as described by Chan and Smith in *Nucl. Acids Res.*, 1984, 12, 2407-2419 or by G. Winter *et al* in *Biochem. Soc. Trans.*, 1984, 12, 224-225.

Recombinant techniques are described in Maniatis *et. al.*, *Molecular Cloning - A Laboratory Manual*; Cold Spring Harbor, 1982-1989.

In particular, a protein or immunogenic derivative for use in the present invention can be prepared using the following steps:

i) preparing a replicable or integrating expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said protein or an immunogenic derivative thereof;

ii) transforming a host cell with said vector;

iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said protein; and

iv) recovering said protein.

The term 'transforming' is used herein to mean the introduction of foreign DNA into a host cell by transformation, transfection or infection with an appropriate plasmid or viral vector using e.g. conventional techniques as described in *Genetic Engineering*; Eds. S.M. Kingsman and A.J. Kingsman;

Blackwell Scientific Publications; Oxford, England, 1988. The term 'transformed' or 'transformant' will hereafter apply to the resulting host cell containing and expressing the foreign gene of interest.

5 The replicable expression vector may be prepared by cleaving a vector compatible with the host cell to provide a linear DNA segment having an intact replicon, and combining said linear segment with one or more DNA molecules which, together with said linear segment encode the desired product, under ligating conditions.

10 Thus, the DNA polymer may be preformed or formed during the construction of the vector, as desired.

The choice of vector will be determined in part by the host cell, which may be prokaryotic or eukaryotic. Suitable vectors include plasmids, bacteriophages, cosmids and recombinant viruses.

15 The preparation of the replicable expression vector may be carried out conventionally with appropriate enzymes for restriction, polymerisation and ligation of the DNA, by procedures described in, for example, Maniatis *et al* cited above.

20 The recombinant host cell is prepared by transforming a host cell with a replicable expression vector under transforming conditions. Suitable transforming conditions are conventional and are described in, for example, Maniatis *et al* cited above, or "DNA Cloning" Vol. II, D.M. Glover ed., IRL Press Ltd, 1985.

25 The choice of transforming conditions is determined by the host cell. Thus, a bacterial host such as *E. coli* may be treated with a solution of CaCl_2 (Cohen *et al*, Proc. Nat. Acad. Sci., 1973, 69, 2110) or with a solution comprising a mixture of RbCl , MnCl_2 , potassium acetate and glycerol, and then with 3-[N-morpholino]-propane-sulphonic acid, RbCl and glycerol. Mammalian cells in culture may be transformed by calcium co-precipitation of the vector DNA onto the cells.

30 Culturing the transformed host cell under conditions permitting expression of the DNA polymer is carried out conventionally, as described in, for example, Maniatis *et al* and "DNA Cloning" cited above. Thus, preferably the cell is supplied with nutrient and cultured at a temperature below 45°C .

35 The product is recovered by conventional methods according to the host cell. Thus, where the host cell is bacterial, such as *E. coli* it may be

lysed physically, chemically or enzymatically and the protein product isolated from the resulting lysate. Where the host cell is mammalian, the product may generally be isolated from the nutrient medium or from cell free extracts. Conventional protein isolation techniques include selective precipitation, absorption chromatography, and affinity chromatography including a monoclonal antibody affinity column.

Preferably, the host cell is E. coli.

A particular aspect of the present invention provides a novel compound which comprises an HCV core protein, or an immunogenic derivative thereof, fused to a polypeptide containing foreign epitopes. The polypeptide is preferably an influenza protein, such as the NS1 protein, or an immunogenic derivative thereof. DNA coding for such a novel compound, vectors containing said DNA, host cells transformed with said vectors, and their use in producing said novel compound, form still further aspects of the invention claimed.

The vaccines of the present invention are preferential stimulators of IgG2a production and TH1 cell response. This is advantageous, because of the known implication of TH₁ response in cell mediated response. Indeed in mice induction of IgG2a is correlated with such an immune response.

The vaccines of the invention enhance induction of cytolytic T lymphocyte responses. Induction of CTL is easily seen when the target antigen is synthesised intracellularly, ie during infection by the virus, because peptides generated by proteolytic breakdown of the antigen can enter the appropriate processing pathway, leading to presentation in association with class I molecules on the cell membrane. However, in general, pre-formed soluble antigen does not reach this processing and presentation pathway, and does not elicit class I restricted CTL. Therefore conventional non-living vaccines, while eliciting antibody and T helper responses, do not generally induce CTL mediated immunity. The combination of the two adjuvants QS21 and 3D-MPL together with an oil in water emulsion can overcome this serious limitation of vaccines based on recombinant proteins, and induce a wider spectrum of immune responses.

In certain systems, the combination of 3D-MPL and QS21 together with an oil in water emulsion have been able to synergistically enhance interferon γ production.

Additionally the oil in water emulsion may contain span 85 and/or lecithin. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in International patent application published under No. 92116556 - SmithKline Beecham Biologicals s.a.

The oil in water emulsion may be utilised on its own or with other adjuvants or immuno-stimulants

In a further aspect of the present invention there is provided a vaccine as herein described for use in medicine.

5 The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1 : 5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5:1 to 1:1 3D MPL: QS21. Typically for human administration QS21 and 3D MPL will be present in a vaccine in the range 1 µg - 100 µg, preferably 10 µg - 50 µg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2
10 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

Vaccine preparation is generally described in New Trends and Developments
15 in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

20 The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Generally, it is expected that each dose will comprise 1-1000 µg of protein, preferably 2-100 µg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses
25 in subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced.

The formulations of the present invention may be used for both prophylactic and therapeutic purposes.

Accordingly in one aspect, the invention provides a method of treatment
30 comprising administering an effective amount of a vaccine of the present invention to a patient.

The following examples illustrate the invention.

Example 1

1.1 Construction and expression of a recombinant HCV core fusion protein

5 Plasmid pMG81 a derivative of pMG27 (Gross et al 1985, Mol.Cell. Biol. 5: 1015) in which: (i) the 81 first codons of the NS1 coding region from influenza strain A/PR/8/34 cleaved from plasmid pAS1EH/801 (Young et al. 1983, Proc. Natl. Acad. Sci. 80: 6105) have been inserted downstream of the pL promoter and ii) the ampicillin resistance gene has been replaced by the kanamycin resistance gene from transposon
10 Tn902, was used to express the fusion protein NS1-Core.

HCV genomic sequences of hepatitis C virus genotype 1b (Delisse et al, 1991 J. Hepathology 13, suppl. 4:S20-23) were PCR amplified and cloned into pUC12 plasmid to give plasmid TCM128-2.

15

The nucleotides sequences corresponding to amino acids 2-166 of the core protein were amplified from TCM128-2. During the polymerase chain reaction, NcoI and XbaI restriction sites have been generated at the 5' and 3' ends of the core sequences allowing insertion into the same sites of plasmid pMG81 to give pRIT 14129.

20

pRIT 14129 contains the coding sequence for the fusion protein NS1 (flu)-core(HCV) and expresses the polypeptide described in SEQ ID NO. 1. The coding sequence for the fusion protein NS1 (flu)-core(HCV) is contained in SEQ ID NO 2. SEQ ID NO 3 shows the amino acid sequence 1-1006 of HCV genome type 1a (H).

25

Plasmid pRIT14129 was introduced into E. coli AR 58 (Mott et al, 1985, Proc, Natl. Acad. Sci., 82:88) containing the thermosensitive repressor of the λ pL promoter.

30

The recombinant bacteria were grown in a 20 Litters fermentor under fed-batch conditions at 30°. The expression of the NS1-Core protein was induced by raising the temperature to 38-42°C. The cells were then harvested and mechanically disrupted.

1.2 Purification of the NS1-Core fusion protein

35 The antigen was purified in a denatured form by preparative electrophoreses:

Step 1: Bacterial cells were broken (Rannie-2 x 14,500 pi) in a 20 mM phosphate buffer pH7 containing protease inhibitors (1mM pefabloc, 0.5mg/leupeptin, 0.1% aprotinin).

- 5 Step 2: Lysate was centrifuged for 25 minutes, at 17,000g. At this stage the recombinant protein was insoluble and was recovered in the pellet. The pellet was washed two times with 10mM phosphate pH6.8, 2M NaCl, 4M urea; three times with 10mM phosphate pH 6.8, 0.15M NaCl, and centrifuged at 17,000g for 25 minutes after each wash step. These steps were introduced in order to lower the endotoxin
10 content of the purified product.

- 15 Step 3: The washed pellets re suspended in SDS-PAGE reducing sample buffer, boiled for 5 minutes, centrifuged again at 27,000g for 25 minutes and then applied on a 12% polyacrylamide gel for separation of the remaining proteins (Prep Cell equipment, Biorad).

- 20 Step 4: The protein was electroluted from the gels in 25mM Tris pH8, 200mM glycine, 0.1% SDS; precipitated by 10% TCA at 0° and finally resuspended in 10mM phosphate pH 6.8, 150mM NaCl, 50mM sarcosyl.

The purified antigen appears as a doublet, in the 27-30 kD range, both bands are recognised by an anti-NS1 monoclonal antibody as well as by anti-core specific human monoclonal and rabbit polyclonal antibodies.

25 1.3 Adjuvantation of the NSI-Core Protein

The two adjuvant formulations were made each comprising the following oil in water emulsion component.

- 30 SB26: 5% squalene 5% tocopherol 0.4% tween 80; the particle size was 500 nm size
SB62: 5% Squalene 5% tocopherol 2.0% tween 80; the particle size was 180 nm

1(a) Preparation of emulsion SB62 (2 fold concentrate)

- 35 Tween 80 is dissolved in phosphate buffered saline (PBS) to give a 2% solution in the PBS. To provide 100 ml two fold concentrate emulsion 5g of DL alpha tocopherol and 5ml of squalene are vortexed to mix thoroughly. 90ml of PBS/Tween solution is added and mixed thoroughly. The resulting emulsion is then passed through a syringe

and finally microfluidised by using an M110S microfluidics machine. The resulting oil droplets have a size of approximately 180 nm.

1(b) Preparation of emulsion SB26

5

This emulsion was prepared in an analogous manner utilising 0.4% tween 80.

1(c) Other emulsions as depicted in the Table were made in an analogous manner.

10 1(d) Preparation of fusion protein/QS21/3D MPL/ oil in water formulation.

To the emulsion of 1 a) or b) or c) an equal volume of twice concentrated fusion protein (either 20µg or 100µg) was added and mixed. This was combined with 50µg/ml of 3D-MPL and 20µg/ml of QS21 to give the final formulation. Buffer was
15 set according to salt content and pH.

Example 2

2.1 Preparation of a recombinant E1E2 oligomeric protein

20

Oligomeric forms of E1-E2 HCV envelope proteins can be prepared from mammalian cells infected with recombinant vaccinia virus expressing HCV envelope sequences as a polyprotein. The coding sequences for a polyprotein covering the amino acids 167-1006 of HCV genome of type 1a (H) can be inserted in vaccinia virus vectors using
25 procedures known in the art and the resulting plasmid used to prepared vaccinia recombinant virus that will lead to expression of the polyprotein in infected cells. The expressed polyprotein is processed and retained intracellularly. E1-E2 oligomeric form can be purified from cell extracts in which the E1/E2 protein complex has been solubilized using specific detergent (Ralston et al, 1993, J. Virology 67:6753)
30 (Dubuisson et al 1994, J. Virology 68:6147).

2.2 Preparation of vaccine formulations

Formulations of oligomeric E1E2 are prepared analagously to the formulations of
35 Example 1.

Example 3

Formulations containing both the fusion protein of Example 1 and the E1E2 oligomer
5 of Example 2 are prepared analogously to the formulations of Example 1, each
formulation containing between 50 and 100µg of each protein.

Table 1Vehicles two fold concentrated

5

Emulsions SB	Tocopherol %	Squalene %	Tween 80 %	Span 85 %	Lecithin %	Size
26	5	5	0.4	0	0	500 nm 90-100% 800 nm 10-0%
26.1	5	5	0.4	0	0.1	500 nm
63	5	5	0.6	0	0	500 nm
64	5	5	0.8	0	0	500 nm
61	5	5	1	0	0	250-300 nm
62	5	5	2	0	0	180 nm
40	5	5	0.4	1	0	500 nm 80-100% 800 nm 20-0%
40.1	5	5	0.4	1	0.1	500 nm
60	5	5	1	1	0	300 nm
65	5	5	0.4	1.5	0	500 nm
66	5	5	0.4	2	0	500 nm

SEQ ID NO 1

1 MDPNTVSSFQ VDCFLWHVRK RVADQELGDA PFLDRLRRDQ KSLRGRGSTL
5 51 GLDIETATRA GKQIVERILK EESDEALKMT MSTNPKPQRK TKRNTNRRPQ
101 DVKFPGGGQI VGGVYLLPRR GPRLGVRATR KTSERSQPRG RRQPIPKARQ
10 151 PEGRAWAQPQ YPWPLYGNEG MGWAGWLLSP RGSRPSWGPT DPRRRSRNLG
201 KVIDTLTCGF ADLMGYIPLV GAPPGGAARA LAHGVRVLED GVNYAT

SEQ ID NO 2

15 1 GAATTCGTAC CTAGATCTCT CACCTACCAA ACAATGCCCC CCTGCAAAAA
51 ATAAATTCAT ATAAAAACA TACAGATAAC CATCTGCGGT GATAAATTAT
20 101 CTCTGGCGGT GTTGACATAA ATACCACTGG CGGTGATACT GAGCACATCA
151 GCAGGACGCA CTGACCACCA TGAAGGTGAC GCTCTTAAAA ATTAAGCCCT
201 GAAGAAGGGC AGCATTCAAA GCAGAAGGCT TTGGGGTGTG TGATACGAAA
25 251 CGAAGCATTG GCCGTAAGTG CGATTCCGGA TTAGCTGCCA ATGTGCCAAT
301 CGCGGGGGGT TTTCGTTTCTAG GACTACAACCT GCCACACACC ACCAAAGCTA
30 351 ACTGACAGGA GAATCCAGAT GGATGCACAA ACACGCCGCC GCGAACGTCG
401 CGCAGAGAAA CAGGCTCAAT GGAAAGCAGC AAATCCCCTG TTGGTTGGGG
451 TAAGCGCAAA ACCAGTTCCG AAAGATTTTT TTAACATAA ACGCTGATGG
35 501 AAGCGTTTAT GCGGAAGAGG TAAAGCCCTT CCCGAGTAAC AAAAAACAA
551 CAGCATAAAT AACCCCGCTC TTACACATTC CAGCCCTGAA AAAGGGCATC
40 601 AAATTAAACC ACACCTTAAG GAGGATATAA CATATGGATC CAAACACTGT
651 GTCAAGCTTT CAGGTAGATT GCTTTCTTTG GCATGTCCGC AAACGAGTTG
701 CAGACCAAGA ACTAGGTGAT GCCCCATTCC TTGATCGGCT TCGCCGAGAT
45 751 CAGAAATCCC TAAGAGGAAG GGCAGCACT CTTGGTCTGG ACATCGAGAC
801 AGCCACACGT GCTGGAAAGC AGATAGTGGA GCGGATTCTG AAAGAAGAAT
50 851 CCGATGAGGC ACTTAAAATG ACATGAGCA CAAATCCTAA ACCCAAAGA
901 AAAACCAAAC GTAACACCAA CCGTCGCCCA CAGGACGTTA AGTTCCTGGG
951 CGGTGGTCAG ATCGTtGGTG GAGTTTACcT GTTGCCGCGC AGGGGCCCCA
55 1001 GGTtGGGTGT GCGcGCGACT AGGAAGACTT CCGAGCGGTC GCAACCTCGT
1051 GGAAGGCGAC AgCCTATCCC CAAGGCTCGC CaGCCCCGAGG GtAGGgCCTG
1101 GGCaCAGCCc GGGTATCCTT GGCCCCCTCTA TGGCAATGAG GGCaTGGGGT
60 1151 GGGCAGGATG GCTCCTGTCA CCCC GCGGCT CcCGGCCTAG TTGGGGCCCC
1201 AcgGACCCCC GGCGTAGGTC GCGTAATTTG GGTAAGGTCA TCGATACCCT
65 1251 cACgTGCGGC TTCGCCGACC TCATGGGGTA CATTCCGCTC GTCGGCGCCC
1301 CCcCAGGGGG CGCTGCCAGG GCctTGGCAC ATGGTGTCCG GGTtCTGGAG

1351 GACGGCGTGA ACTATGCAAC AtaaTCTAGA ATCGATAAGC TTCGACCGAT
5 1401 GCCCTTGAGA GCCTTCAACC CAGTCAGCTC CTTCCGGTGG GCGCGGGGCA
1451 TGACTATCGT CGCCGCACTT ATGACTGTCT TCTTTATCAT GCAACTCGTA
1501 GGACAGGTGC CGGCAGCGCT CTGGGTCATT TTCGGCGAGG ACCGCTTTTCG
10 1551 CTGGAGCGCG ACGATGATCG GCCTGTGCTG TCGCGTATTC GGAATCTTGC
1601 ACGCCCTCGC TCAAGCCTTC GTCAGTGGTC CCGCCACCAA ACGTTTCGGC
1651 GAGAAGCAGG CCATTATCGC CGGCATGGCG GCCGACGCGC TGGGCTACGT
15 1701 CTTGCTGGCG TTCGTCCAGT AATGACCTCA GAACTCCATC TGGATTGTGTT
1751 CAGAACGCTC GGTTGCCGCC GGGCGTTTTT TATTGGTGAG AATCGCAGCA
20 1801 ACTTGTCGCG CCAATCGAGC CATGTCGTCG TCAACGACCC CCCATTCAAG
1851 AACAGCAAGC AGCATTGAGA ACTTTGGAAT CCAGTCCCTC TTCCACCTGC
1901 TGACGACGCG AGGCTGGATG GCCTTCCCCA TTATGATTCT TCTCGCTTCC
25 1951 GGCGGCATCG GGATGCCCCG GTTGCAGGCC ATGCTGTCCA GGCAGGTAGA
2001 TGACGACCAT CAGGGACAGC TTCAAGGATC GCTCGCGGCT CTTACCAGCC
30 2051 TAACTTCGAT CACTGGACCG CTGATCGTCA CGGCGATTTA TGCCGCCTCG
2101 GCGAGCACAT GGAACGGGTT GGCATGGATT GTAGGCGCCG CCCTATACCT
2151 TGTCTGCCTC CCCGCGTTGC GTCGCGGTGC ATGGAGCCGG GCCACCTCGA
35 2201 CCTGAATGGA AGCCGGCGGC ACCTCGCTAA CGGATTCACC ACTCCAAGAA
2251 TTGGAGCCAA TCAATTCTTG CGGAGAACTG TGAATGCGCA AACCAACCCT
40 2301 TGGCAGAACA TATCCATCGC GTCCGCCATC TCCAGCAGCC GCACGCGGCG
2351 CATCTCGGGC AGCGTTGGGT CCTGGCCACG GGTGCGCATG ATCGTGCTCC
2401 TGTGCTTGAG GACCCGGCTA GGCTGGCGGG GTTGCCTTAC TGGTTAGCAG
45 2451 AATGAATCAC CGATACGCGA GCGAACGTGA AGCGACTGCT GCTGCAAAAC
2501 GTCTGCGACC TGAGCAACAA CATGAATGGT CTTCGGTTTC CGTGTTCGT
50 2551 AAAGTCTGGA AACGCGGAAG TCAGCGCCCT GCACCATTAT GTTCCGGATC
2601 TGCATCGCAG GATGCTGCTG GCTACCCTGT GGAACACCTA CATCTGTATT
2651 AACGAAGCGC TGGCATTGAC CCTGAGTGAT TTTTCTCTGG TCCCGCCGCA
55 2701 TCCATACCGC CAGTTGTTTA CCCTCACAAAC GTTCCAGTAA CCGGGCATGT
2751 TCATCATCAG TAACCCGTAT CGTGAGCATC CTCTCTCGTT TCATCGGTAT
60 2801 CATTACCCCC ATGAACAGAA ATTCCCCCTT ACACGGAGGC ATCAAGTGAC
2851 CAAACAGGAA AAAACCGCCC TTAACATGGC CCGCTTTATC AGAAGCCAGA
2901 CATTAAACGCT TCTGGAGAAA CTCAACGAGC TGGACGCGGA TGAACAGGCA
65 2951 GACATCTGTG AATCGCTTCA CGACCACGCT GATGAGCTTT ACCGCAGCTG
3001 CCTCGCGCGT TTCGGTGATG ACGGTGAAAA CCTCTGACAC ATGCAGCTCC

5 3051 CGGAGACGGT CACAGCTTGT CTGTAAGCGG ATGCCGGGAG CAGACAAGCC
3101 CGTCAGGGCG CGTCAGCGGG TGTGCGGGG TGTCGGGGCG CAGCCATGAC
3151 CCAGTCACGT AGCGATAGCG GAGTGTATAC TGGCTTAACT ATGCGGCATC
3201 AGAGCAGATT GTACTGAGAG TGCACCATAT ATGCGGTGTG AAATACCGCA
10 3251 CAGATGCGTA AGGAGAAAAT ACCGCATCAG GCGCTCTTCC GCTTCCTCGC
3301 TCACTGACTC GCTGCGCTCG GTCGTTCGGC TGCGGCGAGC GGTATCAGCT
15 3351 CACTCAAAGG CGGTAATACG GTTATCCACA GAATCAGGGG ATAACGCAGG
3401 AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG
3451 CCGCGTTGCT GCGGTTTTTC CATAGGCTCC GCCCCCTGA CGAGCATCAC
20 3501 AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG
3551 ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA
25 3601 CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGAAGCGTG
3651 GCGCTTCTC AATGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT
3701 TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCCTTCAG CCCGACCGCT
30 3751 GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC
3801 TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA
35 3851 TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA
3901 CTAGAAGGAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTTC
3951 GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG
40 4001 CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA AAAAAAGGAT
4051 CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGAAC
45 4101 GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT
4151 CACCTAGATC CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA
4201 TATATGAGTA AACTTGGTCT GACAGTTACC AATGCTTAAT CAGTGAGGCA
50 4251 CCTATCTCAG CGATCTGTCT ATTTGCTTCA TCCATAGTTG CCTGACTCCC
4301 CGTCGTGTAG ATAACACGA TACGGGAGGG CTTACCATCT GGCCCCAGTG
55 4351 CTGCAATGAT ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA
4401 ATAAACCAGC CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC CTGCAACTTT
4451 ATCCGCCTCC ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA
60 4501 GTTCGCCAGT TAATAGTTTG CGCAACGTTG TTGCCATTGC TGCAGGTCGA
4551 CGGATCAGCC TCGAGGTGAG GTCTGCCTCG TGAAGAAGGT GTTGCTGACT
65 4601 CATACCAGGC CTGAATCGCC CCATCATCCA GCCAGAAAGT GAGGGAGCCA
4651 CGGTTGATGA GAGCTTTGTT GTAGGTGGAC CAGTTGGTGA TTTTGAACCT
4701 TTGCTTTGCC ACGGAACGGT CTGCGTTGTC GGAAGATGC GTGATCTGAT

4751 CCTTCAACTC AGCAAAAGTT CGATTTATTC AACAAAGCCA CGTTGTGTCT
5 4801 CAAAATCTCT GATGTTACAT TGCACAAGAT AAAAATATAT CATCATGAAC
4851 AATAAAACTG TCTGCTTACA TAAACAGTAA TACAAGGGGT GTTATGAGCC
4901 ATATTCAACG GGAAACGTCT TGCTCGAGGC CGCGATTAAA TTCCAACATG
10 4951 GATGCTGATT TATATGGGTA TAAATGGGCT CGCGATAATG TCGGGCAATC
5001 AGGTGCGACA ATCTATCGAT TGTATGGGAA GCCCGATGCG CCAGAGTTGT
5051 TTCTGAAACA TGGCAAAGGT AGCGTTGCCA ATGATGTTAC AGATGAGATG
15 5101 GTCAGACTAA ACTGGCTGAC GGAATTTATG CCTCTTCCGA CCATCAAGCA
5151 TTTTATCCGT ACTCCTGATG ATGCATGGTT ACTCACCCT GCGATCCCCG
20 5201 GGAAACAGC ATTCCAGGTA TTAGAAGAAT ATCCTGATTC AGGTGAAAAT
5251 ATTGTTGATG CGCTGGCAGT GTTCCTGCGC CGGTTGCATT CGATTCCTGT
5301 TTGTAATTGT CCTTTTAAACA GCGATCGCGT ATTCCTCTC GCTCAGGCGC
25 5351 AATCACGAAT GAATAACGGT TTGGTTGATG CGAGTGATTT TGATGACGAG
5401 CGTAATGGCT GGCCTGTTGA ACAAGTCTGG AAAGAAATGC ATAAGCTTTT
30 5451 GCCATTCTCA CCGGATTCAG TCGTCACTCA TGGTGATTTT TCACTTGATA
5501 ACCTTATTTT TGACGAGGGG AAATTAATAG GTTGTATTGA TGTGAGACGA
5551 GTCGGAATCG CAGACCGATA CCAGGATCTT GCCATCCTAT GGAAGTGCCT
35 5601 CGGTGAGTTT TCTCCTTCAT TACAGAAACG GCTTTTTCAA AAATATGGTA
5651 TTGATAATCC TGATATGAAT AAATTGCAGT TTCATTTGAT GCTCGATGAG
40 5701 TTTTTCTAAT CAGAATTGGT TAATTGGTTG TAACACTGGC AGAGCATTAC
5751 GCTGACTTGA CGGGACGGCG GCTTTGTTGA ATAAATCGAA CTTTTGCTGA
5801 GTTGAAGGAT CAGATCACGC ATCTTCCCGA CAACGCAGAC CGTTCCGTGG
45 5851 CAAAGCAAAA GTTCAAAATC ACCAACTGGT CCACCTACAA CAAAGCTCTC
5901 ATCAACCGTG GCTCCCTCAC TTTCTGGCTG GATGATGGGG CGATTGAGG
50 5951 CTGGTATGAG TCAGCAACAC CTTCTTCACG AGGCAGACCT CACCTCGAGG
6001 CTGATCCCCG

SEQ ID NO 3

55 1 MSTNPKPQRK TKRNTNRRPQ DVKFPGGGQI VGGVYLLPRR GPRLGVRATR
51 KTSERSQPRG RRQIPKARR PEGRTWAQPG YPWPLYGNEG CGWAGWLLSP
60 101 RGSRPSWGPT DPRRRSRNLG KVIDTLTCGF ADLMGYIPLV GAPLGGAARA
151 LAHGVRVLED GVNYATGNLP GCSFSIFLLA LLSCLTVPAS AYQVRNSSGL
201 YHVTNDPCNS SIVYEAADAI LHTPGCVPCV REGNASRCWV AVTPTVATRD
65

251 GKLPTTQLRR HIDLLVGSAT LCSALYVGD L CGSVFLVGQL FTFSPRRHWT
301 TQDCNCSTIYP GHITGHRMAW DMMMNWSPTA ALVVAQLLRI PQAIMDMIAG
5 351 AHWGVLAGIA YFSMVGNWAK VLVVLLLFAG VDAETHVTGG NAGRTTAGLV
401 GLLTPGAKQN IQLINTNGSW HINSTALNCN ESLNTGWLAL LFYQHKFNSS
451 GCPERLASCR RLTDFAQGWG PISYANGSGH DERPYCWHYP PRPCGIVPAK
10 501 SVCGPVYCFT PSPVVVGTTD RSGAPTYSWG ANDTDVFLVN NTRPPLGNWF
551 GCTWMNSTGF TKVCGAPPCV IGGVGNNTLL CPTDCFRKHP EATYSRCGSG
15 601 PWITPRCMVD YPYRLWHYPC TINYTIFKVR MYVGGVEHRL EAACNWTRGE
651 RCDLED RDRS ELSPLLLSTT QWQVLPCSFT TLPALSTGLI HLHQNIQDVQ
701 YLYGVGSSIA SWAIKWEYV V LLFLLADAR VCSCLWMMLL ISQAEAALEN
20 751 LVILNAASLA GTHGLVSFLV FFCFAWYLKG RWVPGAVYAL YGMWPLLLLLL
801 LALPQRAYAL DTEVAASCGG VVLVGLMALT LSPYYKRYIS WCMWWLQYFL
25 851 TRVEAQLHWV VPPLNVRGGR DAVILLMCVV HPILVFDITK LLLAIFGPLW
901 ILQASLLKVP YFVRVQGLLR ICALARKIAG GHYVQMAIIK LGALTGTIVY
951 NHLTPLRDWA HNGLRDLAVA VEPVVFSRME TKLITWGADT AACGDIINGL
30 1001 PVSARR

Claims

1. A vaccine composition comprising: QS21; 3 De-O-acylated monophosphoryl lipid A (3D-MPL); an oil in water emulsion, wherein the oil in water emulsion has the
5 following composition: a metabolisable oil, alpha tocopherol and tween 80; and at least one immunogen selected from the group consisting of (a) a hepatitis C virus core protein or an immunogenic derivative thereof, and (b) a hepatitis C virus envelope protein or an immunogenic derivative thereof.
- 10 2. A vaccine composition according to claim 1 wherein the HCV protein or immunogenic derivative thereof is chemically conjugated to a carrier molecule.
3. A vaccine composition according to claim 1 or 2 wherein the immunogenic derivative is a fusion polypeptide.
15
4. A vaccine composition according to claim 3 wherein the fusion polypeptide comprises an HCV core protein or an immunogenic derivative thereof fused to an influenza protein or an immunogenic derivative thereof.
- 20 5. A vaccine composition according to claim 4 wherein the influenza protein is the NS1 protein.
6. A compound which comprises an HCV core protein, or an immunogenic derivative thereof, fused to a polypeptide containing foreign epitopes.
25
7. A compound according to claim 6 wherein the polypeptide containing foreign epitopes is an influenza protein or an immunogenic derivative thereof.
8. A compound according to claim 7 wherein the influenza protein is the NS1
30 protein.
9. A method of treating or preventing HCV infection, which comprises administering to a patient in need thereof an effective amount of a composition according to any one of claims 1 to 5 or a compound according to any one of claims 6
35 to 8.

10. Use of a composition according to any one of claims 1 to 5 or a compound according to any one of claims 6 to 8 in the manufacture of a medicament for use in the prevention or treatment of HCV infection.
- 5 11. A process for the preparation of a composition according to any one of claims 1 to 5, which process comprises mixing the constituents thereof in the required proportions.
- 10 12. A process for the preparation of a compound according to any one of claims 6 to 8, which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.
- 15 13. A DNA molecule encoding a compound according to any one of claims 6 to 8.
14. A recombinant vector comprising the DNA of claim 13.
15. A host cell transformed with the recombinant vector of claim 14.



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/62, A61K 39/29, C07K 14/18	A3	(11) International Publication Number: WO 97/01640 (43) International Publication Date: 16 January 1997 (16.01.97)
(21) International Application Number: PCT/EP96/02764 (22) International Filing Date: 20 June 1996 (20.06.96) (30) Priority Data: 9513261.9 29 June 1995 (29.06.95) GB (71) Applicant (for all designated States except US): SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE). (72) Inventors; and (75) Inventors/Applicants (for US only): CABEZON SILVA, Teresa [CL/BE]; SmithKline Beecham Biological S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). MOMIN, Patricia, Marie [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). GARÇON, Nathalie, Marie-Joséphé, Claude [FR/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). (74) Agent: WEST, Vivien; SmithKline Beecham, Corporate Intellectual Property, SB House, Great West Road, Brentford, Middlesex TW8 9BD (GB).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 15 May 1997 (15.05.97)
(54) Title: VACCINES AGAINST HEPATITIS C		
(57) Abstract <p>A vaccine composition comprises QS21,3 De-O-acylated monophosphoryl lipid A (3D-MPL), an oil in water emulsion, wherein the oil in water emulsion has the following composition: a metabolisable oil, such as squalene, alpha tocopherol and tween 80, and at least one immunogen selected from the group consisting of (a) a hepatitis C virus core protein or an immunogenic derivative thereof, and (b) a hepatitis C virus envelope protein or an immunogenic derivative thereof.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Larvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 96/02764

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/62 A61K39/29 C07K14/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 00153 A (SMITHKLINE BEECHAM BIOLOG ;PRIEELS JEAN PAUL (BE); GARCON JOHNSON) 6 January 1994 see page 5, line 27 - page 6, line 2; claims 1,6 ---	1,2,9-11
X	DATABASE WPI Section Ch, Week 9444 Derwent Publications Ltd., London, GB; Class B04, AN 94-354772 XP002028489 & JP 06 279 500 A (IMMUNO JAPAN KK) , 4 October 1994 see abstract ---	6,9,10, 12-15
X	DE 40 41 304 A (MIKROGEN MOLEKULARBIOLOGISCHE) 25 June 1992 see page 3, line 1 - page 4, line 29 ---	6,9,10, 12-15
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

Date of the actual completion of the international search

27 March 1997

Date of mailing of the international search report

10.04.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Sitch, W

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/EP 96/02764

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 472 207 A (ABBOTT LAB) 26 February 1992 see column 3, line 58 - column 5, line 16 ---	6,9,10, 12-15
X	WO 93 00365 A (CHIRON CORP) 7 January 1993 see page 19, line 16 - page 21, line 16 ---	6,9,10, 12-15
A	WO 93 19780 A (SMITHKLINE BEECHAM BIOLOG ;GARCON JOHNSON NATHALIE MARIE (BE); HAU) 14 October 1993 see page 3, line 19 - page 4, line 23 ---	1,2,9-11
P,X	WO 95 17209 A (SMITHKLINE BEECHAM BIOLOG ;MOMIN PATRICIA MARIE (BE); GARCON NATHA) 29 June 1995 see claims 1,6 ---	1,2,9-11
P,X	WO 95 17210 A (SMITHKLINE BEECHAM BIOLOG ;MOMIN PATRICIA MARIE (BE); GARCON NATHA) 29 June 1995 see claims 1,6 -----	1,2,9-11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 96/02764

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 9
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 9 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 96/02764

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9400153 A	06-01-94	AP 408 A	27-09-95
		AU 661404 B	20-07-95
		AU 4326393 A	24-01-94
		AU 676166 B	06-03-97
		AU 4326493 A	24-01-94
		CA 2138996 A	06-01-94
		CA 2138997 A	06-01-94
		CN 1086142 A	04-05-94
		CN 1092812 A	28-09-94
		CZ 9403296 A	16-08-95
		WO 9400575 A	06-01-94
		EP 0671948 A	20-09-95
		EP 0649470 A	26-04-95
		EP 0761231 A	12-03-97
		FI 946064 A	22-02-95
		HU 71208 A	28-11-95
		JP 7508512 T	21-09-95
		JP 7508648 T	28-09-95
		NO 945003 A	23-12-94
		NZ 253137 A	27-08-96
		NZ 253138 A	26-10-95
		PL 170980 B	28-02-97
		SI 9300335 A	31-12-93
		SK 159294 A	09-08-95
DE 4041304 A	25-06-92	WO 9211370 A	09-07-92
		EP 0564532 A	13-10-93
		JP 8275781 A	22-10-96
		JP 6502542 T	24-03-94
EP 0472207 A	26-02-92	AU 655592 B	05-01-95
		AU 8277491 A	07-05-92
		CA 2049679 A	25-02-92
		JP 4281792 A	07-10-92
WO 9300365 A	07-01-93	AU 671594 B	05-09-96
		AU 2305392 A	25-01-93
		BG 98332 A	28-02-95
		CA 2110058 A	07-01-93
		EP 0591431 A	13-04-94

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 96/02764

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9300365 A		FI 935808 A HU 73098 A JP 6508837 T NO 934542 A	21-02-94 28-06-96 06-10-94 10-02-94
WO 9319780 A	14-10-93	AT 146678 T AU 3751693 A AU 6445796 A CA 2132833 A CN 1085805 A CZ 9402355 A DE 69306940 D EP 0633784 A FI 944442 A HU 69931 A JP 7505372 T NO 943571 A NZ 249868 A SI 9300149 A SK 115294 A	15-01-97 08-11-93 07-11-96 14-10-93 27-04-94 15-02-95 06-02-97 18-01-95 26-09-94 28-09-95 15-06-95 14-11-94 24-03-97 31-12-93 07-06-95
WO 9517209 A	29-06-95	AU 1316495 A AU 1316695 A CA 2179779 A CN 1138298 A WO 9517210 A EP 0735898 A ZA 9410176 A	10-07-95 10-07-95 29-06-95 18-12-96 29-06-95 09-10-96 17-11-95
WO 9517210 A	29-06-95	AU 1316495 A AU 1316695 A CA 2179779 A CN 1138298 A WO 9517209 A EP 0735898 A ZA 9410176 A	10-07-95 10-07-95 29-06-95 18-12-96 29-06-95 09-10-96 17-11-95

THIS PAGE BLANK (USPTO)